Journal of Chromatography, 426 (1988) 438–443
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4101

Note

High-performance liquid chromatographic assay of *trans*-(−)-5,5a,6,7,8,9,9a,10-octahydro-6-propylpyrimido[4,5-*g*]quinolin-2-amine dihydrochloride (LY163502), a potent D-2 agonist, in plasma

B.J. MILLER*

Department of Drug Disposition, Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285 (U.S.A.)

J.B. TENBARGE

Department of Medical Information, Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285 (U.S.A.)

and

J.R. BERNSTEIN and R.B. FRANKLIN

Department of Drug Disposition, Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285 (U.S.A.)

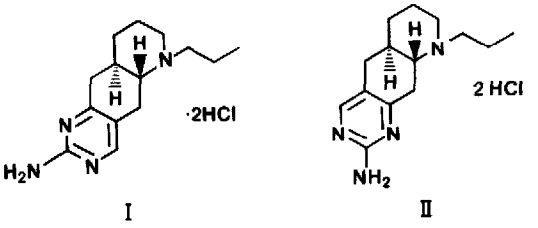
(First received October 12th, 1987; revised manuscript received December 18th, 1987)

Compound LY163502 (I; *trans*-(−)-5,5a,6,7,8,9,9a,10-octahydro-6-propylpyrimido[4,5-*g*]quinolin-2-amine dihydrochloride; Fig. 1), a partial ergoline structure, is a specific and potent dopamine (D-2) receptor agonist [1–5] being investigated by Lilly Research Labs. Assays of biological fluids to determine parent drug concentrations are important in the development of any pharmaceutical product. This paper describes a high-performance liquid chromatographic (HPLC) assay used for determination of plasma levels of I in preclinical pharmacology and toxicology studies in rats.

EXPERIMENTAL

Reagents and standards

All reagents were of analytical or HPLC grade. Glycine–sodium hydroxide buffer was prepared by combining 80 ml of 0.2 *M* glycine with 125 ml of 0.2 *M* sodium hydroxide, diluting to 500 ml with water, and adjusting the pH to 10.0. Compound I and the internal standard, II (LY173818; *trans*-(±)-



I II

Fig. 1. Chemical structures of I and II (internal standard).

5,5a,6,7,8,9,9a,10-octahydro-9-propylpyrimido[5,4-*g*]quinolin-2-amine dihydrochloride (see Fig. 1), were synthesized in the Lilly Research Labs. (Indianapolis, IN, U.S.A.). Solutions of I and II used for dosing and standards were prepared in water.

HPLC system

A Waters Model 6000A HPLC pump system connected to a WISP 710B autoinjector (Waters) equipped with a fluorescence detector (Perkin-Elmer LS-5 or Schoeffel FS 970) was used. The excitation wavelength for the detector was 230 nm and the emission wavelength was 360 nm. Separation was obtained using a Whatman Partisil 5 ODS 3 RAC column (10 cm × 9.4 mm I.D., 5 μm particle size) protected by a Brownlee RP-8 or RP-18 LiChrosorb pre-column (3 cm × 4.6 mm I.D., 10 μm particle size) and a spherical silica guard column, the latter being placed between the pumps and the WISP. The mobile phase consisted of methanol–water (60:40, v/v) with 10 m*M* triethylamine in the methanol. Mobile phase was prepared fresh daily. The HPLC system was operated at ambient temperature and with a solvent flow-rate of 2 ml/min.

Plasma extraction procedure

Internal standard (20–25 μl of an aqueous solution containing 10 μg/ml II) was added to 1 ml of plasma. Proteins were precipitated by the addition of acetone (1.3 ml) followed by freezing in an acetone–solid carbon dioxide bath. The sample was allowed to thaw at room temperature, then spun in a centrifuge at approximately 800–2000 *g* for 10 min. The supernatant was decanted into a tube containing 2 ml glycine–sodium hydroxide buffer, pH 10. Ethyl acetate (5 ml) was added and the mixture was shaken vigorously for 10 min, then spun in a centrifuge for 10 min at approximately 800–2000 *g*. The upper organic phase was transferred to another tube and the solvent was removed in vacuo or under a stream of dry nitrogen. The residue was dissolved in approximately 200 μl of mobile phase and an aliquot was injected onto the HPLC system via the WISP autoinjector.

Standard curves

Standard curves were generated by adding known amounts of I and internal standard (II) to control rat plasma. The plasma was then extracted as above. Concentrations of I generally ranged from 25 to 800 ng/ml of plasma. Chroma-

tograms were acquired and processed using a HP-1000 chromatography system (Hewlett-Packard) and Lilly software (Eli Lilly & Co.). A least-squares analysis of the concentration versus peak-area ratios (I/II) was used to calculate the calibration line, which in turn was used to calculate the concentration of I in plasma samples. In some applications, peak-height ratios were used in calculations.

Precision and accuracy

Intra-day and inter-day precisions were established at four different concentrations (25, 150, 600 or 1600 ng/ml I in plasma) by adding I and internal standard to blank plasma followed by extraction and analysis as described above. Five replicates of each concentration were assayed on each of three days by the same analyst on the same liquid chromatograph.

Plasma samples

As one example of the use of this assay, data are presented from a kinetic study with rats. Male Sprague–Dawley rats (Charles River) were dosed orally or intravenously (tail vein) with I at 20 mg/kg. Animals were sacrificed at 0 (pre-dose), 0.5, 1, 2, 4, 6, 8, 12 or 24 h after dosing ($n=3$). In addition, rats dosed intravenously were also sacrificed at 0.25 h. Blood was collected in heparinized tubes and plasma was obtained by centrifugation (approximately 800 *g* for 15 min). Ali-

TABLE I

SUMMARY OF PRECISION AND ACCURACY DATE FOR I

Day		Standard I			
		25 ng/ml	150 ng/ml	600 ng/ml	1600 ng/ml
Intra-assay					
1	Mean (ng/ml)	34.87	153.12	644.74	1690.25
	S.D. (ng/ml)	2.11	11.72	28.95	39.52
	n	4	5	5	4
	R.S.D. (%)	6.04	7.65	4.49	2.34
2	Mean (ng/ml)	18.48	153.03	647.55	1497.20
	S.D. (ng/ml)	3.84	8.81	13.85	34.74
	n	5	5	4	5
	R.S.D. (%)	20.78	5.76	2.14	2.32
3	Mean (ng/ml)	25.90	156.40	643.22	1727.80
	S.D. (ng/ml)	4.98	6.05	13.88	53.25
	n	4	5	5	5
	R.S.D. (%)	19.22	3.87	2.16	3.08
Inter-assay					
	Mean (ng/ml)	25.81	154.18	645.00	1634.71
	R.S.D. (%)	36.06	6.11	3.44	8.37
	Total variance (%)	74.00	0	0	93.48
	Percentage of theoretical	103.23	102.79	107.50	102.17

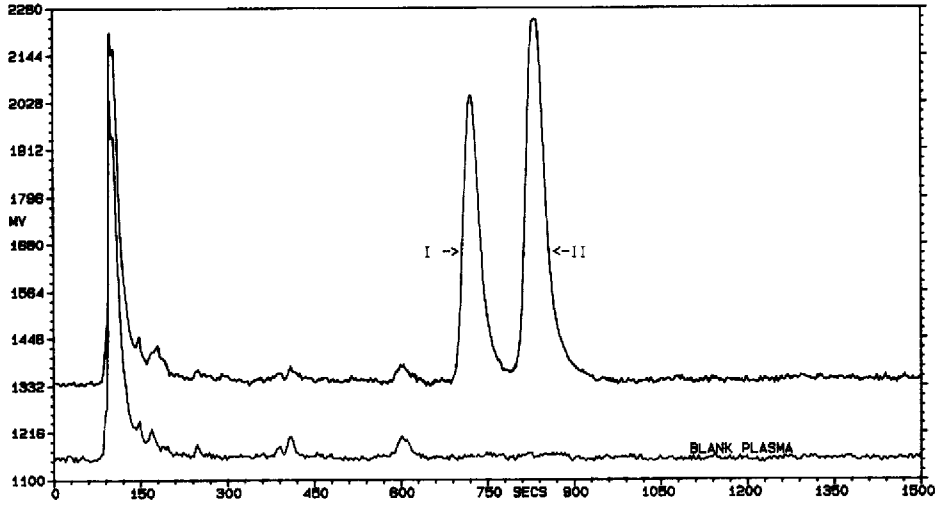


Fig. 2. Chromatograms of extracted control rat plasma (lower trace) and rat plasma spiked with I (500 ng) and II (500 ng) (upper trace), extracted and assayed by HPLC as described in the Experimental section.

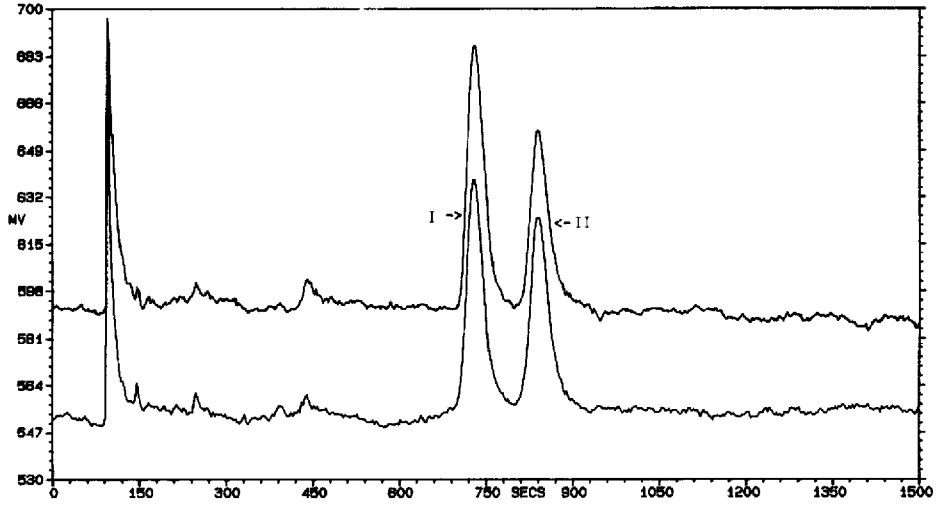


Fig. 3. Chromatograms of an extract of rat plasma assayed 1 h (lower trace) or 2 h (upper trace) after the oral administration of 40 mg I per kg body weight, containing 1.6 and 2.1 μg/ml, respectively.

quots (1 ml) of the plasma were assayed as described above. Areas under the curve (AUCs) were calculated using a trapezoidal method. Systemic bioavailability was calculated from AUC values as follows: bioavailability= $(AUC_{p.o.}/AUC_{i.v.}) \times 100\%$.

RESULTS

Standard curves, precision and accuracy

The useful detection limit for this assay is approximately 20 ng/ml of plasma. Precision and accuracy data are summarized in Table I. At the 25 ng/ml concentration (near the detection limit), the coefficient of variation (C.V.) for I ranged

TABLE II

COMPARATIVE PLASMA LEVELS OF MALE SPRAGUE-DAWLEY RATS DOSED ORALLY AND INTRAVENOUSLY WITH I AT 20 mg/kg

Assay sensitive to 25 ng/ml of plasma. N.D. = not detected.

Time (h)	Concentration of I in plasma (mean ± S.D., $n=3$) (μg/ml)	
	Orally	Intravenously
0	N.D.	N.D.
0.25	–	24 ± 0.8
0.5	0.407 ± 0.109	25 ± 2.3
1	0.628 ± 0.185	16 ± 3.3
2	0.495 ± 0.121	3 ± 0.4
4	0.190 ± 0.024	1.5 ± 0.1
6	0.431 ± 0.139	1.5 ± 0.3
8	0.364 ± 0.078	1.3 ± 0.3
12	0.224 ± 0.012	0.17 ± 0.08
24	0.250 ± 0.093	0.17 ± 0.03
Area under the curve (0–24 h)	7.045	43.648
$t_{\frac{1}{2}}$ (0.5–4 h)	–	Approximately 50 min

from 6.04 to 20.78% for intra-day and was 13.42% for inter-day. At the other concentrations, the coefficients of variation ranged from 2.14 to 7.65% for intra-day and 0.13 to 1.76% for inter-day.

Plasma samples

Chromatograms obtained from extracted blank plasma and extracted plasma to which had been added I and internal standard are shown in Fig. 2. Chromatograms from plasma of rats dosed orally with I are shown in Fig. 3. Table II lists the concentrations of I in rat plasma over a 24-h period after an oral or intravenous dose of 20 mg/kg. After an initial decline, plasma levels of I plateaued from 4 to 8 h after the intravenous dose and, after the oral dose even increased between 4 and 6 h after dosing. This phenomenon has been observed in other rat studies and is attributed to redistribution of the compound. AUC values for the 0–24 h period were 7.045 and 43.648 μg h/ml for oral and intravenous administration, respectively. Systemic bioavailability was thus calculated to be 16%. Results from tissue distribution studies (data not shown) indicate that this apparent low value represents a distribution rather than an absorption phenomenon.

DISCUSSION

The HPLC assay with fluorescence detection reported here has proven satisfactory for monitoring plasma levels as low as 20 ng/ml. Plasma levels greater than the limits of the standard curve can be accurately determined by diluting the plasma with physiologic saline and extracting a 1-ml aliquot of diluted plasma. In addition to the application illustrated in this paper, this assay has been used

in preclinical pharmacology and toxicology studies in rats, mice and dogs. A further development has been the elaboration of an enzyme linked immunosorbent assay (ELISA) by Taber et al. [6] which can measure plasma levels of I between 10 and 300 pg/ml.

REFERENCES

1 F.P. Bymaster, D.T Wong, P.G. Threlkeld, L.R. Reid, C.L. Nichols and E.C. Kornfeld, Pharmacologist, 26 (1984) 218.
2 F.P. Bymaster, L.R. Reid, C.L. Nichols, E.C. Kornfeld and D.T. Wong, Life Sci., 38 (1986) 317.
3 M.M. Foreman and J.L. Hall, J. Neural Transm., 68 (1987) 153.
4 M.M. Foreman and J.L. Hall, Psychopharmacology, 91 (1987) 96.
5 E.B. Smalstig and J.A. Clemens, Pharmacologist, 26 (1984) 136.
6 L.D. Taber, J.M. Apathy, A.F. DeLong and J.R. Sportsman, J. Pharm. Sci., 76 (1987) 492